



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : C07H 21/00, 21/02		A1	(11) International Publication Number: WO 00/69878
			(43) International Publication Date: 23 November 2000 (23.11.00)
(21) International Application Number: PCT/US00/13777 (22) International Filing Date: 19 May 2000 (19.05.00) (30) Priority Data: 09/314,603                      19 May 1999 (19.05.99)                      US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US    09/314,603 (CON) Filed on                                      19 May 1999 (19.05.99)		(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71)(72) Applicant and Inventor: NGO, Nam, Q. [VN/US]; 4191 Rincon Avenue, Campbell, CA 95008 (US). (74) Agents: MOOI, Leslie A. et al.; Burns, Doane, Swecker & Mathis, LLP, P.O. Box 1404, Alexandria, VA 22313 (US).		Published With international search report.	
(54) Title: UNIVERSAL SOLID SUPPORTS SUITABLE FOR THE SYNTHESIS OF NUCLEIC ACIDS			
(57) Abstract			
<p>Novel Universal Solid Supports suitable for use synthesizing of oligonucleotides. The solid supports may be used irrespective of the first RNA or DNA nucleotide to be synthesized, and irrespective of the type of monomer reagent used during the synthesis, that is, irrespective of the type of substitution of the phosphate group in the 3' position or in the 5' position depending on whether the synthesis is carried out in the 5' to 3' or 3' to 5' direction. Following synthesis of the oligonucleotide, deprotection of protecting groups and cleavage of the oligonucleotide from the solid support is accomplished with treatment with standard basic media such as NH<sub>4</sub>OH, NaOH, Methylamine..., etc.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<b>AL</b>	Albania	<b>ES</b>	Spain	<b>LS</b>	Lesotho	<b>SI</b>	Slovenia
<b>AM</b>	Armenia	<b>FI</b>	Finland	<b>LT</b>	Lithuania	<b>SK</b>	Slovakia
<b>AT</b>	Austria	<b>FR</b>	France	<b>LU</b>	Luxembourg	<b>SN</b>	Senegal
<b>AU</b>	Australia	<b>GA</b>	Gabon	<b>LV</b>	Latvia	<b>SZ</b>	Swaziland
<b>AZ</b>	Azerbaijan	<b>GB</b>	United Kingdom	<b>MC</b>	Monaco	<b>TD</b>	Chad
<b>BA</b>	Bosnia and Herzegovina	<b>GE</b>	Georgia	<b>MD</b>	Republic of Moldova	<b>TG</b>	Togo
<b>BB</b>	Barbados	<b>GH</b>	Ghana	<b>MG</b>	Madagascar	<b>TJ</b>	Tajikistan
<b>BE</b>	Belgium	<b>GN</b>	Guinea	<b>MK</b>	The former Yugoslav Republic of Macedonia	<b>TM</b>	Turkmenistan
<b>BF</b>	Burkina Faso	<b>GR</b>	Greece			<b>TR</b>	Turkey
<b>BG</b>	Bulgaria	<b>HU</b>	Hungary	<b>ML</b>	Mali	<b>TT</b>	Trinidad and Tobago
<b>BJ</b>	Benin	<b>IE</b>	Ireland	<b>MN</b>	Mongolia	<b>UA</b>	Ukraine
<b>BR</b>	Brazil	<b>IL</b>	Israel	<b>MR</b>	Mauritania	<b>UG</b>	Uganda
<b>BY</b>	Belarus	<b>IS</b>	Iceland	<b>MW</b>	Malawi	<b>US</b>	United States of America
<b>CA</b>	Canada	<b>IT</b>	Italy	<b>MX</b>	Mexico	<b>UZ</b>	Uzbekistan
<b>CF</b>	Central African Republic	<b>JP</b>	Japan	<b>NE</b>	Niger	<b>VN</b>	Viet Nam
<b>CG</b>	Congo	<b>KE</b>	Kenya	<b>NL</b>	Netherlands	<b>YU</b>	Yugoslavia
<b>CH</b>	Switzerland	<b>KG</b>	Kyrgyzstan	<b>NO</b>	Norway	<b>ZW</b>	Zimbabwe
<b>CI</b>	Côte d'Ivoire	<b>KP</b>	Democratic People's Republic of Korea	<b>NZ</b>	New Zealand		
<b>CM</b>	Cameroon			<b>PL</b>	Poland		
<b>CN</b>	China	<b>KR</b>	Republic of Korea	<b>PT</b>	Portugal		
<b>CU</b>	Cuba	<b>KZ</b>	Kazakstan	<b>RO</b>	Romania		
<b>CZ</b>	Czech Republic	<b>LC</b>	Saint Lucia	<b>RU</b>	Russian Federation		
<b>DE</b>	Germany	<b>LI</b>	Liechtenstein	<b>SD</b>	Sudan		
<b>DK</b>	Denmark	<b>LK</b>	Sri Lanka	<b>SE</b>	Sweden		
<b>EE</b>	Estonia	<b>LR</b>	Liberia	<b>SG</b>	Singapore		

## UNIVERSAL SOLID SUPPORTS SUITABLE FOR THE SYNTHESIS OF NUCLEIC ACIDS

This application claims the benefit of provisional application serial number 60/088,507  
5 filed on June 8<sup>th</sup>, 1998 and application serial number 60/086,041 filed on May 19, 1998.

### FIELD OF THE INVENTION

The present invention relates generally to the synthesis of nucleic acids and more  
particularly relates to solid supports that are useful in automated synthesis of DNA or RNA  
10 oligonucleotides.

### BACKGROUND FO THE INVENTION

Universal Solid supports used in automated oligonucleotide synthesis posses pre-attached  
nucleosides to provide a chain initiation site for oligonucleotide construction. Chain elongation  
15 occurs by sequential addition of monomeric phosphoramidite unites on the 5' hydroxyl. During  
the deprotection process of the oligodeoxyribonucleotide, the pre-attached nucleoside is cleaved  
from the solid support and retained o the oligodeoxyribonucleotide as the 3'-terminated base.  
Solid supports suitable for automated synthesis of oligonucleotides must satisfy the following  
characteristics:

- 20 1) the solid support must react selectively with the functionalized 3' end of the  
nucleotide in particular of the phosphoramidite, H-phosphonate, phosphotriester,  
phosphodiester, phosphite type or with any other monomer reagent according to  
the synthetic method used;
- 25 2) the support-oligonucleotide bond must be stable under the conditions of the  
synthesis, and
- 3) the support-oligonucleotide bond must be able to be hydrolyzed at the end of the  
synthesis under the conditions for the step of deprotection of the oligonucleotide,  
and
- 30 4) the covalent bond between the support and oligonucleotide must be such that,  
during the detachment, the released oligonucleotide must be such that, during the  
detachment, the release oligonucleotide is of native type, that is to say that the 3'  
terminal hydroxyl function is free or does not bear any residue derived from the  
synthesis.

Many supports have already been described in the literature for the solid phase synthesis of oligonucleotides. These supports may consist of organic polymers such a polystyrene (Nucleic A. Res. 1980, Vol. 8), polyacrylamide acryloylmorpholide, polydimethyl acrylamide polymerized on kieselguhr (Nucleic Acid Res. 9(7) 1691(1980)). Other supports described are of inorganic nature, in particular based on silica functionalized with a hydrocarbon radical bearing an NH<sub>2</sub> and/or COOH group (JACS, 105, 661 (1983), or the support based on silica functionalized with a 3-aminopropyltriethoxysilane group whose use in phosphite and phosphoramidite synthesis for the preparation of oligonucleotides described for the first time in European patent No. 0,035,719.

Typically, when employing prior art solid supports, the first step in synthesizing nucleic acids consists of attaching the first nucleoside of the desired sequence to the solid support, traditionally consisting of glass beads of controlled porosity (CPG) or, more generally, of a functionalized organic or inorganic polymer bound to an A, T, C, G or U nucleoside, depending on whether the sequence to be prepared contains A, T, C, G, or U as the first deoxyribo- or ribonucleotide. Thus, commercially available automated reactors are equipped so that one of these nucleosides has already been attached to the support. The appropriate reactor is thus selected depending on whether the sequence begins with A, T, C, G, or U. Elongation of this first nucleoside then takes place in the 3' to 5' or 5' to 3' direction, by means of coupling reagents. One synthetic cycle, that is to say the coupling between two nucleotides, includes at least three steps: (1) deprotection of the 5' or 3' OH function of a first nucleotide, e.g., detritylation, (2) activation of the said 5' or 3' OH function of this first nucleotide and condensation with the 3' or 5' end respectively of a second nucleotide, and, finally, (3) oxidation of the phosphite group of the internucleotide bond obtained to phosphate.

The oligonucleotide is preferably synthesized in the 3' → 5' direction. In this case, the starting material is a 5' OH-protected nucleoside that is attached to the support via the 3' end of the deoxyribose or ribose ring. The nucleotides which are subsequently added are in the form of a 5'-protected derivative whose 3' hydroxyl possesses a substituted phosphite or phosphate group.

Various synthetic methods are currently employed and they are distinguished by the type of substitution on the phosphate. The phosphoramidite method, described, for example, in EP 61,746 and US 4,458,066, is the preferred technique because of the high coupling yields achieved. In this method, a phosphoramidite group is attached to the 3' hydroxyl. Besides the importance of these groups for the solubility of the nucleosides in the organic solvent, the phosphoramidite group renders the phosphorus atom more susceptible to attack by a primary

hydroxyl function, such as that in the 5' position of the detritylated growing nucleosides or chains. The deprotected 5' hydroxyl function becomes sufficiently nucleophilic to react with the phosphoramidite group of the second nucleotide.

5 The oligonucleotides obtained at the end of the synthetic cycles must be detached from the support and the protective functions must be removed. Cleavage of the support, deprotection of the bases and removal of the group bonded to the phosphorus are carried out simultaneously in aqueous ammonia solution. In the case of RNA, ethanol makes it possible to solubilize the 2'-O-silyl-oligoribonucleotides, and to minimize the desilylation as native RNA is not stable in basic conditions. The aqueous ammonia/ethanol solution containing the oligoribonucleotide  
10 which has passed into the liquid phase is then separated from the glass support and evaporated. Removal of the silyl groups takes place in the presence of tetrabutylammonium fluoride (TBAF) at room temperature for sixteen hours. The TBAF is then neutralized with TEAA (triethylammonium acetate). Other methods include, for example, the so-called phosphotriester, phosphodiester, H-phosphonate, and phosphite methods.

15 As is apparent, despite the advantages that have been achieved in DNA and RNA syntheses, the art is in need of a multifunctional solid support that could be used to synthesize any oligonucleotide regardless of the nature of the 3'-terminal base.

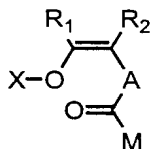
### SUMMARY OF THE INVENTION

20 The present invention is based, in part, on the development of universal solid supports suitable for use synthesizing of oligonucleotides. The solid supports are universal in that they may be used irrespective of the first RNA or DNA nucleotide to be synthesized, and irrespective of the type of monomer reagent used during the synthesis, that is, irrespective of the type of substitution on the phosphate group in the 3' position or in the 5' position depending on whether  
25 the synthesis is carried out in the 5' to 3' or 3' to 5' direction.

The novel solid supports of the present invention can be employed in automated solid phase syntheses using standard process conditions. In particular, with the present invention, the monomer reagent serving to attach the first nucleotide to the solid support should be a monomer reagent identical to the monomer reagent serving to attach the other nucleotides of the sequence  
30 during the synthesis, in particular as regards the 5' protection and the 3' protection.

With the present invention, the first nucleotide that is introduced contains a 3' or 5' phosphate group which is, after cleavage between the support and the oligonucleotide, under the usual conditions of deprotection in basic medium, capable at the end of the synthesis of liberating an end 3' or 5' OH. In one aspect, the invention is directed to multifunctional

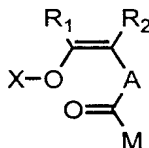
substrates suitable as a reagent for synthesizing oligonucleotide acids have the following formulae:



One feature of the invention is that following synthesis of the oligonucleotide, deprotection of the protecting groups and cleavage of the oligonucleotide from the solid support is accomplished with treatment with standard basic medium such as: (NH<sub>4</sub>OH, NaOH...).

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Substrates suitable as a reagent for synthesizing oligonucleotide acids have the following formulae:



R<sub>1</sub>, R<sub>2</sub>: alkyl, cycloalkyl, heteroaryl, aryl, and heterocyclic. R<sub>1</sub> and R<sub>2</sub> may be fused to form an aryl ring (preferably a phenyl ring), heteroaryl ring, heterocyclic ring or cycloalkyl ring, heterocyclic ring or cycloalkyl ring.

X: H or Z (protecting group)

- Z: the protecting group can be acid labile, base labile or photolabile.
- Acid labile: some are cited here for reference but not limited to: trityl, monomethoxytrityl, dimethoxytrityl
- Base labile: some are listed here for reference but not limited to: 9-fluorenylmethyl chloroformate.
- Photolabile: some are listed here for reference but not limited to: m-nitrophenylcarbamate.

A: Heteroatom: O, N, S.

Where M is represented by L-W, where W is preferably a solid substrate (organic and /or inorganic) such as controlled pore glass (CPG), alkylamine CPG, wherein alkyl can be from 1 to 50 carbon atoms, and isomeric forms thereof, any chemical modifications of CPG, wherein the modification can be, for example, amines, hydroxyls, carboxyls, sulfhydryls, or disulfides, copolymers of styrene and divinylbenzene and any solid support stable to all the conditions of solid phase oligonucleotide synthesis, W can also be a non-solid phosphoramidite group, -OP(OR<sub>c</sub>)NR<sub>4</sub>R<sub>5</sub>, wherein R<sub>c</sub> is -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>CN, or alkane of 1 to 50 carbon atoms, inclusive, and isomeric forms thereof, and R<sub>4</sub>, R<sub>5</sub> is methyl, ethyl, isopropyl, or alkane as defined above (if W is a non-solid phosphoramidite group, it is not restricted to the 3' terminus), and X is a cleavable linking arm connecting carbon C to W which can be any combination of atom groups, (e.g., -CO(CH<sub>2</sub>)<sub>N</sub>-, -CO<sub>2</sub>-, -CONH-, -COS-) that covalently connects to the solid phase (W) through a cleavable linkage, and is stable to all the conditions of solid phase oligonucleotide synthesis. Cleavable linkages include esters, amides, thioester, carbamate, and carbonate, which are cleaved with ammonium hydroxide. After oligonucleotide synthesis, cleavage of X from the solid phase results in the transfer of the entire multifunctional linking arm to the 3' terminus of the synthesized oligonucleotide. R<sub>1</sub>, R<sub>2</sub> are independently selected from H or an inert group. The term "inert group" refers here to a group that does not react under the conditions encountered during the various steps of the synthesis according to the invention of nucleic acids on a solid support. The inert group preferably is, for example, an alkyl group, which is optionally substituted, in particular with one or more halogen(s),

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having a single cyclic ring or multiple condensed rings that can be optionally substituted with from 1 to 3 alkyl groups. Such cycloalkyl groups include, by way of example, methylcyclopropyl, 2-methylcyclopentyl, 2-methylcyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

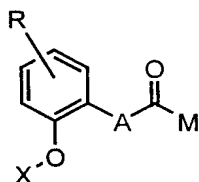
"Heteroaryl" refers to a monovalent aromatic carbocyclic group of from 2 to 8 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within the ring. Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 3 substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, halo, nitro, heteroaryl, thioalkoxy, thioaryloxy and the like. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyll or benzothionyl). Preferred heteroaryls include pyridyl, pyrrol and furyl.

"Heterocyclo" or "heterocyclic" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 8 carbon atoms and from 1 to 4 heteroatoms

selected from nitrogen, sulfur or oxygen within the ring. Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 3 substituents selected from the group consisting of alkyl, alkoxy, aryl, aryloxy, halo, nitro, heteroaryl, thioalkoxy, thioaryloxy and the like. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heteroaryls include morpholine, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholine, piperidinyl, tetrahydrofuranyl, and the like.

In a preferred embodiment, the substrates have the following formulae:



R: -H, any withdrawal electron group as: -CN, -NO<sub>2</sub>, -CO-R<sub>1</sub>, -CONH-R<sub>1</sub>, F, Cl, Br, I

Alkyl or aryl group.

Any electron donor group as: -OH, -OR, -NH<sub>2</sub>, -NHR

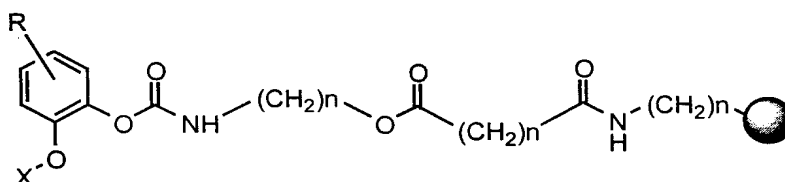
-R<sub>1</sub>: H, alkyl, and aryl

M: same definition as above

X: same definition as above



In a particularly preferred embodiment, the substrate has the structure:



- 5  
R: H, -OCH<sub>3</sub>, -CONH-R<sub>1</sub>, -CO<sub>2</sub>R<sub>1</sub>, -COR<sub>1</sub>  
N: 1 to 12  
X: Trityl, monomethoxytrityl, dimethoxytrityl  
● : Solid Support

10 The inventive substrates can be employed to synthesize nucleic acids, in particular the automatic synthesis, using conventional techniques. The inventive solid support, can especially be exploited for the manufacture of oligonucleotides modified at the terminal 3' end by using directly, in the first cycle, monomers corresponding to the desired nature of the modification.

15 The term "nucleic acid" refers to deoxyribonucleic acids or ribonucleic acids or, more generally, polynucleotides or oligonucleotides in which the bases, internucleotide phosphate bonds or the ribose rings of the bases may be chemically modified in a known manner. They may in particular be oligonucleotides of alpha or beta anomers, oligonucleotides of internucleotidic bonding of the phosphorothioate or methyl phosphonate type, or alternatively oligothionucleotides.

20 In one particular embodiment, the process of the invention comprises the following steps of:

- 1) condensation of the 5' or 3' OH group of the first nucleotide or of an oligonucleotide connected at its other 3' or 5' end to the said solid support, using a coupling agent, with the phosphate group optionally substituted in the 3' or 5' position, respectively, of a nucleotide monomer reagent protected in the 3' and 5' positions;
- 25 2) oxidation or sulfurization of the internucleotide bond of the phosphite type obtained in step 1) to a phosphate bond, respectively;
- 3) deprotection of the 5' -O or 3' -O end of the product obtained in step 2);

- 4) repetition of steps 1) to 3) as many times as there are nucleotides to be added in order to synthesize the nucleic acid.

More precisely, the process may comprise the following steps of:

- 5 1) condensation, using a coupling agent, of the said SH group of the said solid support with a phosphate or phosphite group optionally substituted in the 3' or 5' position of a nucleotide monomer reagent protected in the 5'-O and 3'-O position;
- 2) oxidation or sulfurization of the covalent bond of the phosphite type between the solid support and the first nucleotide obtained in step 1);
- 3) deprotection of the 5'-O or 3'-O end of the product obtained in step 2);
- 10 4) condensation of the 5'OH or 3'OH group of the product obtained in step 3) with the phosphate, phosphorothioate or phosphite group optionally substituted in the 3' or 5' position of a nucleotide monomer reagent protected in the 5'-O or 3'-O position, respectively, using the said coupling agent, under the same conditions as in step 1);
- 15 5) oxidation or sulfurization of the internucleotide grouping of the phosphite type resulting from the above step into a grouping of the phosphate or phosphorothioate type, respectively;
- 6) deprotection of the 5'-O or 3'-O end of the product obtained in step 5);
- 7) repetition of steps (4), (5) and (6) as many times as there are nucleotides to be added in order to obtain the nucleic acid to be prepared.
- 20

The above steps lead to an oligonucleotide connected to the solid support. In an appropriate manner, the process according to the invention includes a final step of detachment of the nucleic acid from the support and removal of the protecting groups from the bases and, where appropriate, from the 2'-O positions of the nucleic acid.

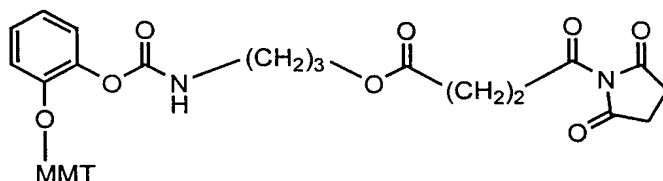
25 In the prior techniques in which the solid support is already connected to a first nucleoside corresponding to the first nucleotide of the sequence to be prepared, before starting the synthetic cycles, the said support generally contains a protection of the said nucleoside in the 5' or 3' position. In this case, the synthetic cycle begins with a step of deprotection in acid medium, generally a detritylation with TCA, DCA or TCA in dichloromethane.

30

Synthesis of Inventive Substrates

It should be noted that only the main reaction products are shown. The reactions were conducted at ambient temperature (about 22°C) and were monitored by TLC.

Synthesis of:



5

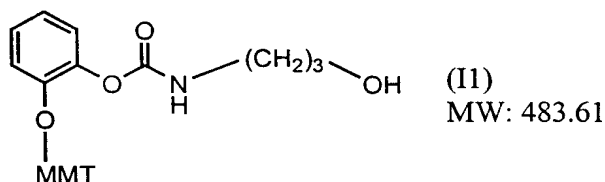
a/ 110mg (1mmol) of catechol was dissolved in 3 ml of tetrahydrofuran (THF) and 140 umol (1mmol) of triethylamine (TEA). 309 mg (1mmol) of monomethoxytrityl chloride was added slowly to the solution and allowed to react for about 30 min. at room temperature. The salt (TEA.HCl) formation was filtered and the crude solution is used as it is for the next step.

10

b/ Added 85 ul (1mmol) of pyridine (1mmol) to the filtrate solution and 202 mg (1mmol) of paranitrophenylchloroformate (PNCF) was added to the solution and it was kept under stirring for 30 min. at room temperature. Filtered the salt (pyridine HCl) formed and used the filtrate solution for the next step.

15

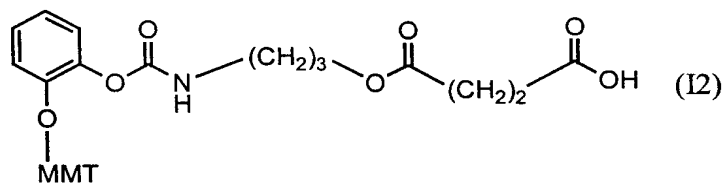
c/ Added 76.5 ul of 3-aminopropanol followed by addition of 140 ul of TEA (1mmol). The reaction solution was agitated for 12 hours at room temperature. After the agitation, evaporated the solvent then purified the crude by flash chromatography to obtain the desired intermediate (I1):



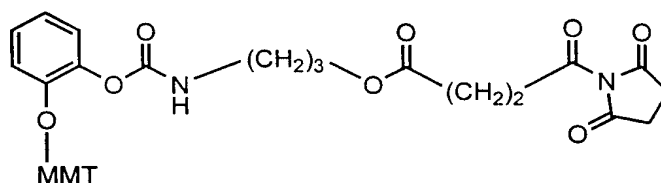
20

Yield: 250mg, 52%

d/ Dissolved 242mg of (I1) (0.5mmol) in 5 ml of acetonitrile. 50 mg of succinic anhydride (0.5 mmol) and 40 ul of pyridine was added to the solution and allowed to react overnight. The product was co-evaporated with acetonitrile (2x). The following intermediate (I2) was obtained with 100% yield.



e/ Dissolved the obtained (I2) in 5 ml of Acetonitrile and 57.5 mg (0.5 mmol) of N-hydroxysuccinimide was added to the solution and allowed to react for about 2 hours. The urea was filtered and the solution was evaporated to give a pure final product with 100 % yield:



WM: 680.61

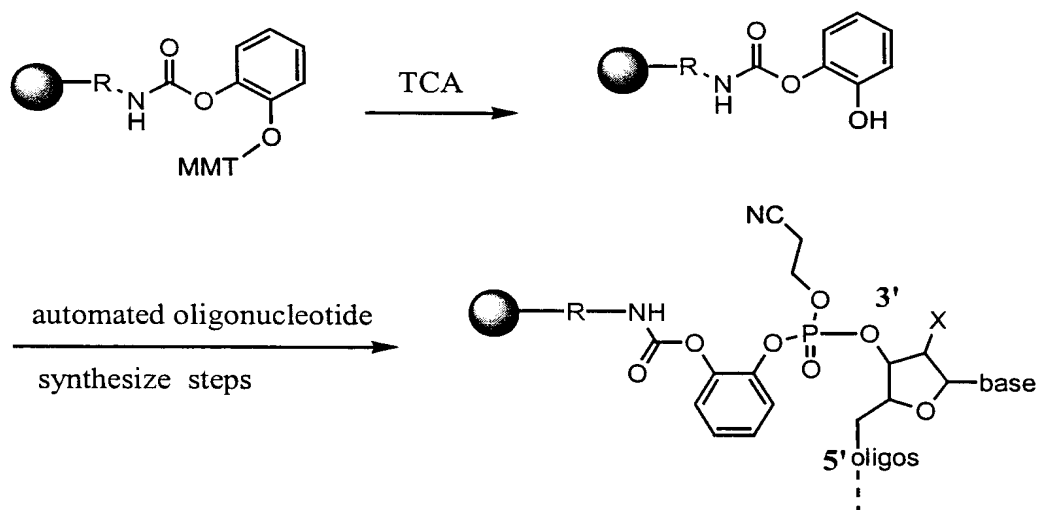
The desired product was used to derivatize the CPG with aminolinker arm on c) as follow:

-1 g of CPG-NH<sub>2</sub> is added to the solution made of 5ml of acetonitrile, 140 mg product (200μmol), 100 ul of triethylamine and the reaction was allowed to proceed for 3 hours at ambient temperature

#### Synthesis of Oligonucleotide

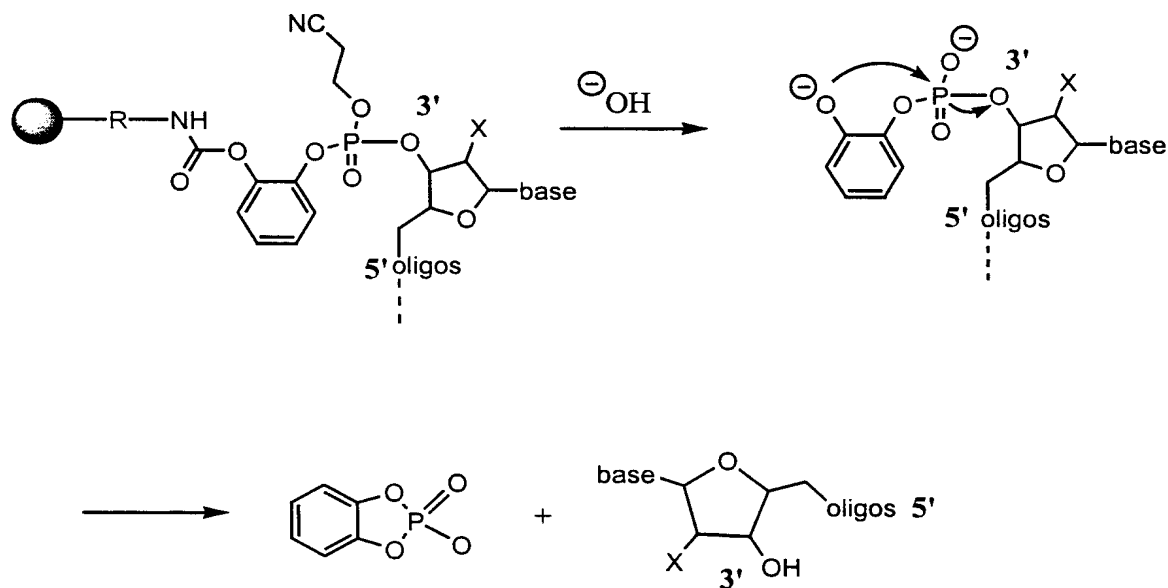
The following scheme illustrates oligodeoxyribonucleotide synthesis by the phosphoramidite method with the inventive substrate using standard conditions.

Phosphoramidite chemistry is well known and is described in US Patents 4,725,677; 4,458,066; 4,415,732, WO 95/10987, and BioTechniques Vol. 22, No. 4 pp 752-756, 1997, which are incorporated herein:



The synthesized oligonucleotides is then cleaved and deprotected with basic media as (NH<sub>4</sub>OH), leaving a 3'OH end suitable for any use as shown below.

5



: Solid support (silica gel or polymeric substrate)

R: linker which is a hydrocarbon chain, containing CHNO

DMT: dimethoxytrityl

Base: A= Adenine

G= Guanine

10

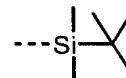
11

C= cytosine

T= Thymine

U= uracil

X: H or OR<sub>1</sub> with R<sub>1</sub> is hydrocarbon structure (C, H, N, O) and/or



5

Although only preferred embodiments of the invention are specifically disclosed and described about, it will be appreciated that many modifications and variations of the present invention are possible in light of the about teachings and within the preview of the appended claims without departing from the spirit and intended scope of the invention.

10

15

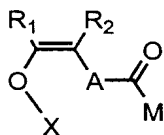
20

25

30

What is claimed is:

1. A substrate that is suitable for synthesizing oligonucleotides that is represented by the following formulae:



R<sub>1</sub>, R<sub>2</sub>: alkyl, cycloalkyl, heteroaryl, aryl, heterocyclic. R<sub>1</sub> and R<sub>2</sub> may be fused to form an aryl ring (preferably a phenyl ring), heteroaryl ring, heterocyclic ring or cycloalkyl ring, heterocyclic ring or cycloalkyl ring.

X: H or Z (protecting group)

- Z: the protecting group can be acid labile, base labile or photolabile.
- Acid labile: some are cited here for reference but not limited to: trityl, monomethoxytrityl, dimethoxytrityl
- Base labile: some are listed here for reference but not limited to: 9-fluorenylmethyl chloroformate.
- Photolabile: some are listed here for reference but not limited to: m-nitrophenylcarbamate.

A: Heteroatom: O, N, S.

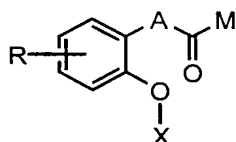
Where M is represented by L-W, where W is preferably a solid substrate (organic and /or inorganic) such as controlled pore glass (CPG), alkylamine CPG, wherein alkyl can be from 1 to 50 carbon atoms, and isomeric forms thereof, any chemical modifications of CPG, wherein the modification can be, for example, amines, hydroxyls, carboxyl's, sulfhydryls, or disulfides, copolymers of styrene and divinylbenzene and any solid support stable to all the conditions of solid phase oligonucleotide synthesis, W can also be a non-solid phosphoramidite group, -OP(OR<sub>c</sub>)NR<sub>4</sub>R<sub>5</sub>, wherein R<sub>c</sub> is -CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>CN, or alkane of 1 to 50 carbon atoms, inclusive, and isomeric forms thereof, and R<sub>4</sub>, R<sub>5</sub> is methyl, ethyl, isopropyl, or alkane as defined above (if W is a non-solid phosphoramidite group, it is not restricted to the 3' terminus), and X is a cleavable linking arm connecting carbon C to W which can be any combination of atom groups, (e.g., -CO(CH<sub>2</sub>)<sub>N</sub>-, -CO<sub>2</sub>-, -CONH-, -COS-) that covalently connects to the solid phase (W) through a cleavable linkage, and is stable to all the conditions of solid phase oligonucleotide

synthesis. Cleavable linkages include esters, amides, thioester, carbamate, and carbonate, which are cleaved with ammonium hydroxide. After oligonucleotide synthesis, cleavage of X from the solid phase results in the transfer of the entire multifunctional linking arm to the 3' terminus of the synthesized oligonucleotide. R<sub>1</sub>, R<sub>2</sub> are independently selected from H or an inert group.

- 5 The term "inert group" refers here to a group that does not react under the conditions encountered during the various steps of the synthesis according to the invention of nucleic acids on a solid support. The inert group preferably is, for example, an alkyl group, which is optionally substituted, in particular with one or more halogen(s),

10

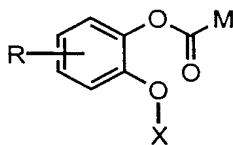
2. The substrate of claim 1, wherein R<sub>1</sub> and R<sub>2</sub> are fused to form a ring.
3. The substrate of claim 2 wherein the ring is preferably a phenyl ring is represented by the formulae:



15

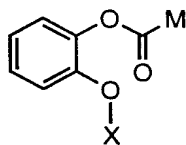
R: H, alkyl, aryl, electron withdrawing groups, electron donor groups

4. The substrate of claim 3 wherein said substrate is represented by the formulae:



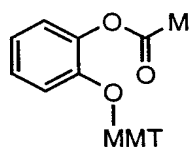
20

5. The substrate of claim 4 wherein said substrate is represented by the formulae:

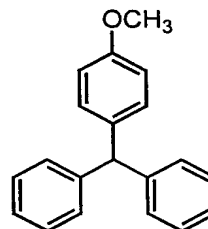




6. Process for the preparation of a nucleic acid by synthesis on a substrate having a solid support, wherein the substrate is represented by the following formulae:

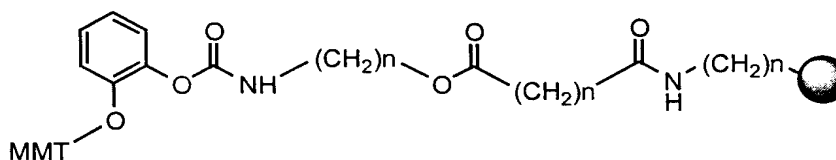


MMT:



Where M is represented by L-W, where W is preferably a solid substrate (organic and/or inorganic) such as controlled pore glass (CPG), alkylamine CPG, wherein alkyl can be from 1 to 50 carbon atoms, and isomeric forms thereof, any chemical modifications of CPG, wherein the modifications of CPG, wherein the modification can be, for example, amines, hydroxyls, carboxyls, sulfhydryls, or disulfides, copolymers of styrene and divinylbenzene and any solid support stable to all the conditions of solid phase oligonucleotide synthesis, W can also be a non-solid phosphoramidite group,  $-OP(OR_3)NR_4R_5$ , wherein  $R_3$  is  $-CH_3$ ,  $CH_2CH_2CN$ , or alkane of 1 to 50 carbon atoms, inclusive, and isomeric forms thereof, and  $R_4, R_5$  is methyl, ethyl, isopropyl, or alkane as defined above (if W is a non-solid phosphoramidite group, it is not restricted to the 3' terminus), and X is a cleavable linking arm connecting carbon C to W which can be any combination of atom groups that covalently connects to the solid phase (W) through a cleavable linkage, and is stable to all the conditions of solid phase oligonucleotide synthesis.

7. The process of claim 6 wherein said substrate is represented by the formulae:



n: 1 to 12

8. The process of claim 7 characterized in that the first nucleotide is advantageously attached to the solid support under the same conditions and with the same monomer reagent as for the condensation of the second nucleotide with the first nucleotide bonded to the support, which may be the conventional conditions and monomer reagent used during the synthesis of

nucleic acids on a solid support, the said first nucleotide corresponding to the first nucleotide in the sequence to the said nucleic acid.

9. The process of claim 7 characterized in that it comprises the following steps of:

- a) condensation of the 5' or 3' OH group of the first nucleotide or of an oligonucleotide connected at its other 3' or 5' end to the said solid support, using a coupling agent, with the phosphate group optionally substituted in the 3' or 5' position respectively of a monomer nucleotide reagent protected in the 3' and 5' positions;
- b) oxidation or sulfurization of the internucleotide bond of the phosphite type obtained in step a) to a phosphate or phosphorothioate bond, respectively;
- c) deprotection of the 5' -O or 3'-O end of the product obtained in step b);
- d) repetition of step a) to c) as many times as there are nucleotides to be added in order to synthesize the nucleic acid.

10. Process of Claims 7 characterized in that it comprises the following steps of:

- a) condensation, using a coupling agent, of the said OH group of the solid support with a phosphate or phosphite group optionally substituted in the 3' or 5' position of a monomer nucleotide reagent protected in the 5'-O and 3-O positions;
- b) oxidation or sulfurization of the covalent bond of the phosphite type between the solid support and the first nucleotide obtained in step a);
- c) deprotection of the 5'-O or 3'-O end of the product obtained in step b);
- d) condensation of the 5'OH or 3'OH group of the product obtained in step c) with the phosphate, phosphorothioate or phosphite group optionally substituted in the 3' or 5' position of a monomer nucleotide reagent protected in the 5'-O or 3'-O position, respectively, using the said coupling agent, under the same conditions as the condensation in step a);
- e) oxidation or sulfurization of the internucleotide grouping of the phosphite type resulting from the above step into a grouping of the phosphate or phosphorothioate type, respectively;
- f) deprotection of the 5'-O or 3'-O end of the product obtained in step e);
- g) repetition of steps, (d), (e) and (f) as many times as there are nucleotides to be added in order to obtain the nucleic acid to be prepared.

11. The process of claim 10 characterized in that it includes a final step of detachment of the nucleic acid from the support and removal of the protecting groups from the bases and, where appropriate, from the 2'-O positions of the nucleic acids.

5

12. The process of claim 11 wherein detachment and removal of the protecting groups is accomplish with treatment with basic media as:  $\text{NH}_4\text{OH}$ ,  $\text{NaOH}$ , Methylamine....

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/13777

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : C07H 21/00, 21/02 US CL : 536/25.3, 25.31 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/25.3, 25.31 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAS ONLINE, WEST		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,458,066 A (CARUTHERS et al.) 03 July 1984, see entire patent document.	1-12
A	EP 0 061 746 A (UNIVERSITY PATENTS, INC.) 10 June 1982, see entire document.	1-12
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 01 AUGUST 2000		Date of mailing of the international search report 18 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer BENNETT CELSA Telephone No. (703) 308-0196

**DERWENT-ACC-NO:** 2001-070763

**DERWENT-WEEK:** 200113

*COPYRIGHT 2008 DERWENT INFORMATION LTD*

**TITLE:** New supports useful for making  
oligonucleotides

**INVENTOR:** NGO N Q

**PATENT-ASSIGNEE:** NGO N Q[NGONI]

**PRIORITY-DATA:** 1999US-314603 (May 19, 1999)

**PATENT-FAMILY:**

<b>PUB-NO</b>	<b>PUB-DATE</b>	<b>LANGUAGE</b>
WO 0069878 A1	November 23, 2000	EN
AU 200052760 A	December 5, 2000	EN

**DESIGNATED-STATES:** AE AG AL AM AT AU AZ BA BB BG  
BR BY CA CH CN CR CU CZ DE DK  
DM DZ EE ES FI GB GD GE GH GM  
HR HU ID IL IN IS JP KE KG KP  
KR KZ LC LK LR LS LT LU LV MA  
MD MG MK MN MW MX MZ NO NZ PL  
PT RO RU SD SE SG SI SK SL TJ  
T M TR TT TZ UA UG US UZ VN YU  
ZA ZW AT BE CH CY DE DK EA ES  
FI FR GB GH GM GR IE IT KE LS  
LU MC MW MZ NL OA PT SD SE SL  
SZ TZ UG ZW

**APPLICATION-DATA:**

<b>PUB-NO</b>	<b>APPL-DESCRIPTOR</b>	<b>APPL-NO</b>	<b>APPL-DATE</b>
WO2000069878A1	N/A	2000WO- US13777	May 19, 2000
AU 200052760A	Based on	2000AU- 052760	May 19, 2000

**INT-CL-CURRENT:****TYPE**

CIPS

**IPC DATE**

C07H21/00 20060101

**RELATED-ACC-NO:** 2003-743006**ABSTRACTED-PUB-NO:** WO 0069878 A1**BASIC-ABSTRACT:**

NOVELTY - Substrates for making oligonucleotides (I) are new.

DESCRIPTION - Substrates for making oligonucleotides of formula (I) are new:

R<sub>1</sub>, R<sub>2</sub> = (cyclo)alkyl, (hetero)aryl, heterocyclo or R<sub>1</sub>+R<sub>2</sub> are (hetero)aryl (preferably phenyl), heterocyclo or cycloalkyl;

X = hydrogen (H) or Z;

Z = acid-labile, base-labile or photo-labile protecting group;

A = oxygen (O), nitrogen (N) or Sulfur (S)  
heteroatoms;

M = L-W;

W = solid substrate (preferably (1-50C alkylamine)  
controlled pore glass optionally modified with  
amines, hydroxyls, carboxyls, sulfhydryls,  
disulfides, styrene copolymers or divinylbenzene)  
or a non-solid phosphoramidite, OP(ORc)NR<sub>4</sub>R<sub>5</sub> or 1-  
50C alkyl;

Rc = methyl or CH<sub>2</sub>CH<sub>2</sub>CN;

R<sub>4</sub>, R<sub>5</sub> = (m)ethyl, isopropyl or other 1-50C alkyl  
(if W is non-solid phosphoramidite it is not  
restricted to the 3' terminus);

X = cleavable linking arm covalently connecting C  
to W that is stable to all solid phase  
oligonucleotide synthesis conditions where the  
link is entirely cleavable with ammonium  
hydroxide; and

R<sub>1</sub>, R<sub>2</sub> = H or inert group especially alkyl  
optionally substituted with one or more halo.

An INDEPENDENT CLAIM is made for a process for  
making a nucleic acid on a solid support substrate  
of formula (II) or (III):

MMT = compound (III).

Cleavable support for oligonucleotide synthesis.

USE - Useful for making nucleic acids, especially  
for the automated synthesis of DNA or RNA

oligonucleotides.

ADVANTAGE - The novel supports are universal for any initial nucleotide regardless of whether the phosphate is in the 3' or 5' position.

## **EQUIVALENT-ABSTRACTS:**

### ORGANIC CHEMISTRY

Synthesis: No general method of synthesis is disclosed.

### PHARMACEUTICALS

Preferred Compounds: Compound of formula (IV) is preferred:

$n = 1-12$ .

Preferred Method: The first nucleotide is preferably attached to the support.

Oligonucleotides are made by either:

(1) the following procedure:

(a) condensing the 5' or 3' hydroxyl of the first nucleotide to the solid support with a coupling agent with the phosphate optionally substituted in the 3' or 5' position of a monomer nucleotide reagent protected in the 3' or 5' positions;

(b) oxidizing or sulfurizing the internucleotide phosphite bond to a phosphate/phosphorothioate; and

(c) deprotecting the 3'-O or 5'-O end of the product and (d) repeating (a)-(c) as required; or



(2) the following procedure:

(a) condensing the hydroxyl of the solid support with a coupling agent using a phosphate/phosphite group optionally substituted in the 3' or 5' position of a monomer nucleotide protects in the 5'-O or 3'-O positions;

(b) oxidizing or sulfurizing the covalent phosphite bond;

(c) deprotecting the 3'-O or 5'-O end of the product;

(d) condensing the 5' or 3' hydroxyl of the product with a coupling agent with the phosphate, phosphothioate or phosphite optionally substituted in the 3' or 5' position of a monomer nucleotide reagent protected in the 3' or 5' positions;

(e) oxidizing or sulfurizing the internucleotide phosphite bond to a phosphate/phosphorothioate;

(f) deprotecting the 3'-O or 5'-O end of the product; and

(g) repeating (d)-(f) as required. The nucleotide is then detached from the support and deprotected, preferably with a base e.g. ammonium hydroxide, sodium hydroxide or methylamine.

#### SPECIFIC COMPOUNDS

The use of WM680.61 is disclosed.

Catechol (1 mmol) was dissolved in tetrahydrofuran (3 ml) and triethylamine (1 mmol) before reacting with monomethoxytrityl chloride (1 mmol) for 30

minutes. The filtered solution was added to pyridine (1 mmol) and the mix was reacted with paranitrophenylchloroformate (1 mmol) for 30 minutes. The filtered solution was reacted with 3-aminopropanol (76.5 mul) and triethylammonium (1 mmol) under agitation for 12 hours. The solvent was evaporated and the crude was purified by flash chromatography. The purified intermediate(242 g) was dissolved in acetonitrile (5 ml) before adding succinic anhydride (0.5 mmol) and pyridine (40 mul) and reacting overnight. After co-evaporating with acetonitrile twice, a second intermediate was dissolved in acetonitrile (5 ml) and reacted with N-hydroxysuccinimide (0.5 mmol) for 2 hours to give WM680.61 (100 % yield).

**TITLE-TERMS:** NEW SUPPORT USEFUL

**DERWENT-CLASS:** B05

**CPI-CODES:** B04-B03C; B05-B01P; B07-H; B10-D03; B10-G01; B10-G02;

**CHEMICAL-CODES:** Chemical Indexing M1 \*05\*  
Fragmentation Code M720 N209 N262  
N309 N381 N511 N512 N513 N514  
N515 Specific Compounds RA013I  
Registry Numbers 184610

Chemical Indexing M2 \*01\*  
Fragmentation Code B415 B515 B615  
B701 B711 B720 B731 B819 B831  
F010 F019 F020 F021 F029 G010  
G019 G020 G021 G029 G030 G039  
G040 G050 G100 G111 G221 G299  
G553 G563 H7 H721 J011 J012 J290  
L432 L450 L463 M121 M122 M123

M124 M125 M126 M133 M210 M211  
M212 M213 M214 M215 M216 M220  
M221 M222 M223 M224 M225 M226  
M231 M232 M233 M273 M280 M281  
M282 M312 M313 M314 M315 M316  
M321 M331 M332 M333 M342 M343  
M344 M373 M383 M391 M411 M413  
M414 M415 M416 M510 M520 M521  
M522 M530 M531 M532 M540 M541  
M542 M710 Markush Compounds  
003100201

Chemical Indexing M2 \*02\*  
Fragmentation Code B415 B515 B615  
B701 B711 B720 B731 B819 B831  
F010 F019 F020 F021 F029 G010  
G019 G020 G021 G029 G030 G039  
G040 G050 G100 G111 G221 G299  
G553 G563 J011 J012 J290 J5 J581  
L432 L450 L463 M121 M122 M123  
M124 M125 M126 M135 M210 M211  
M212 M213 M214 M215 M216 M220  
M221 M222 M223 M224 M225 M226  
M231 M232 M233 M262 M273 M280  
M281 M282 M311 M312 M313 M314  
M315 M316 M321 M331 M333 M340  
M342 M343 M349 M371 M381 M391  
M411 M413 M414 M415 M416 M510  
M520 M521 M522 M530 M531 M532  
M540 M541 M542 M620 M710 Markush  
Compounds 003100202

Chemical Indexing M2 \*03\*  
Fragmentation Code B615 B701 B711  
B720 B731 B819 B831 F021 G050  
J011 J012 J290 L432 L450 L463

M210 M211 M212 M213 M214 M215  
M216 M220 M221 M222 M223 M224  
M225 M226 M231 M232 M233 M273  
M280 M281 M282 M320 M411 M413  
M510 M521 M530 M540 M710 Markush  
Compounds 003100203

Chemical Indexing M2 \*04\*  
Fragmentation Code G010 G011 G015  
G019 G100 H5 H542 H8 J0 J012 J2  
J271 J3 J371 K0 L4 L463 L6 L640  
M1 M121 M129 M132 M141 M150 M210  
M211 M272 M281 M311 M312 M313  
M314 M315 M316 M321 M322 M323  
M332 M342 M343 M382 M383 M391  
M414 M510 M520 M533 M540 M710  
Markush Compounds 003100204

**SECONDARY-ACC-NO:**

**CPI Secondary Accession Numbers:** 2001-019650